

Family association studies of markers on chromosome 2q and Type 1 diabetes in subjects from South India

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Abstract

Background Several Type 1 diabetes susceptibility loci have been located to chromosome 2q12–21. However, results have not always been consistent and this may reflect study design and the population analysed. We have used a family-based design to look for an association between Type 1 diabetes and markers located to 2q12–21.

Methods Ninety-one South Indian families consisting of subjects with Type 1 diabetes and their parents were genotyped for eight polymorphic markers localised to 2q12–21, which includes the interleukin-1 gene cluster. Radiation hybrid mapping was used to localise the map position of D2S308 and D2S363 on 2q12–21. The extended transmission disequilibrium test was used for statistical analysis.

Results No associations were found between Type 1 diabetes and markers located in and around the interleukin-1 gene cluster or the interleukin-1 Type 1 receptor. In contrast, a suggestive association was found between Type 1 diabetes and two closely-linked markers telomeric of the interleukin-1 gene cluster (D2S308 and D2S363, separated by 3.3 cR) ($p=0.004$ and $p=0.002$, respectively).

Conclusion This preliminary study suggests that a locus close to D2S308 and D2S363 is involved in the aetiology of Type 1 diabetes in the South Indian population. Copyright © 2000 John Wiley & Sons, Ltd.

Keywords chromosome 2; Type 1 diabetes; genetic susceptibility; South Indian

Introduction

Many chromosomal regions have been linked to Type 1 diabetes susceptibility in humans, using a variety of approaches including candidate gene testing, genome scanning and human homologue studies of mouse susceptibility genes [1]. The largest contribution from a single locus comes from several genes located in the MHC complex on chromosome 6p21 (*IDDM1*), which account for a large proportion of the heritability to Type 1 diabetes. Human chromosome 2q is also particularly rich in putative diabetic susceptibility loci including *IDDM7* [2–5], *IDDM12* [4–7] and *IDDM13* [4,8].

The chromosome 2q12–22 region contains several immunomodulatory genes including the interleukin-1 and interleukin-1 receptors gene clusters. The interleukin-1 (IL-1) gene cluster contains the genes for interleukin 1 α (IL1A), interleukin 1 β (IL1B) and the interleukin-1 receptor antagonist

(IL1RN) on an approximately 430 kb segment on chromosome 2q12–13 [9–11]. Inconsistent associations have been reported between Type 1 diabetes and this cluster [5,12–14]. Centromeric to the IL-1 gene cluster, on 2q12 is the interleukin-1 receptor (IL-1R) cluster covering 530 kb which contains the genes for the type II interleukin-1(IL-1) receptor (IL1R2), the type IIL-1 receptor (IL1R1), the IL-1 receptor-related protein 2 (IL1RL2), T1/ST2/fit-1 (IL1RL1) and a component of interleukin 18 receptor (IL18R1) [15]. An association between the type 1 interleukin-1 receptor genotype (IL1RI) and Type 1 diabetes has been demonstrated using case control studies in Danish, British and Finnish Caucasians [14,16,17]. However these observations have not been replicated in several other ethnic groups including South Indian and Spanish [17,18].

In view of these results we looked for susceptibility loci for Type 1 diabetes in chromosome 2q12–21 in simplex South Indian families, using polymorphic markers from this region. We report a suggestive association with microsatellite markers telomeric to the IL-1 gene clusters but not the IL-1 gene cluster itself.

Methods

A total of 91 South Indian families, each consisting of an affected subject with Type 1 diabetes diagnosed before the age of 35 years and both parents, were recruited from the Diabetic Research Centre and the MV Hospital for Diabetes in Chennai (formerly Madras). All patients had acute onset of diabetes, were life long insulin-dependent, and all had suffered from episodes of ketonuria or diabetic ketoacidosis. The mean age of disease onset was 10.1 years (± 6.8), the male to female ratio 0.44, and the mean body mass index at the time of venesection 18.2 (± 5.1). Cases of fibrocalculous pancreatic diabetes (FCPD) were excluded [19]. Amongst the fathers, 20.5% had Type 2 diabetes and 3% Type 1 diabetes, whilst in the mothers, 5% had Type 2 diabetes. Informed consent was obtained from all family members before venous sampling and blood samples were obtained from both parents and affected offspring.

We analysed several polymorphic markers from chromosome 2q12–21 in these 91 simplex families to determine whether a susceptibility gene was located in this chromosomal region in this ethnic group.

DNA typing

DNA was extracted from blood samples using Puregene kits (Gentra Systems Inc., Minneapolis, MN, USA). Primers for eight microsatellite markers (Table 1) located to 2q12–21 (sequences obtainable from the Genome Database) were synthesised, fluorescently labelled (Applied Bio-Perkin Elmer, Foster City, CA, USA) and used to amplify the DNA. PCR products were run on a 6% polyacrylamide gel (Flowgen, Ashby Park, Leicestershire,

Table 1. Extended transmission disequilibrium test (ETDT) of chromosome 2q12–21 markers

Marker	TDT <i>p</i> value (<i>n</i> =91)
D2S1473 (IL1R1)	0.09
IL1R1 (RFLP)	0.22
D2S160	0.12
IL1A	0.40
D2S121	0.15
D2S308	0.004
D2S363	0.002
D2S410	0.45

UK) using an ABI 373 sequencer and analysed with Genescanner (Foster City, CA, USA) software. In addition, a PCR-RFLP was used to study IL1R1 as previously described [17]. Briefly, PCR products were digested with *Pst*I, electrophoresed in a 3% agarose gel, and visualised by ethidium bromide staining.

Building of marker map

The Genebridge-4 release of the radiation hybrid panel [supplied by the Human Genome Mapping Project, Medical Research Council (MRC), Cambridge, UK] was amplified using the D2S363 and D2S308 microsatellite primers, and the products separated on denaturing polyacrylamide sequencing gels containing 6% acrylamide and 7 M urea. Results from the screening were entered into the STS mapping programme at the Whitehead Institute's World Wide Web site (<http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>) as a continuous vector of '1' or '0', depending on whether amplification was observed or not. Hybrids 4BB12, 4A5, 4BB10, 4U3, 4B9, 4O10, 4B2, 4R12 and 4K8 were not supplied by the MRC and so results for these were entered in their appropriate positions in the vector as '2' (unknown). The LOD score for acceptance of positioning onto the Framework map was set at 19. In this area of the genome, a distance of 1 cR on the radiation hybrid map is approximately equivalent to 300 kb [20].

Statistical analysis

The extended transmission disequilibrium test (ETDT) [21] was used to identify alleles preferentially transmitted from heterozygous parents to diabetic offspring. Since we analysed eight markers, two pairs of which were in tight linkage disequilibrium, allele-wise *p* values (*p*_{corr}) were corrected by a factor of six. Data from D2S363 and D2S308 were combined in these South Indian subjects to form haplotypes, and were analysed using a modified version of ETDT, which assesses statistical significance using Monte-Carlo methods to produce empirical *p* values. Pairwise linkage disequilibrium (LD) between markers was assessed using the estimate haplotype frequencies (EH) programme [22,23].

Table 2. Significance levels for linkage disequilibrium between pairs of markers on 2q12–21

	IL1R1 ^a	D2S160	IL1A	D2S121	D2S308	D2S363	D2S410
D2S1473	$p < 0.00001$	$p = 0.57$	$p = 0.36$	$p = 0.10$	$p = 0.56$	$p = 0.14$	$p = 0.68$
IL1R1 ^a		$p = 0.69$	$p = 0.34$	$p = 0.11$	$p = 0.10$	$p = 0.10$	$p = 0.73$
D2S160			$p = 0.007$	$p = 0.22$	$p = 0.38$	$p = 0.27$	$p = 0.90$
IL1A				$p = 0.13$	$p = 0.74$	$p = 0.70$	$p = 0.79$
D2S121					$p = 0.29$	$p = 0.67$	$p = 0.52$
D2S308						$p = 0.94$	$p = 0.87$
D2S363							$p = 0.38$

^a*Pst*I RFLP.

Significance levels were assessed using the estimate haplotype frequencies (EH) programme.

Results

The IL1 cluster was investigated initially using three markers (D2S160, D2S363 and IL1R1) (Table 1) in the South Indian families. The only marker which produced a significant result on allele-wise ETDT analysis was D2S363 ($p = 0.0019$; $p_{\text{corr}} 0.01$); this was largely accounted for by excess transmission of allele 3 (257 mu), which was transmitted 46 times and not transmitted 19 times ($p = 0.0008$). In light of this positive finding, a further five markers from the region were then studied: IL1A (GDB157015), D2S121, D2S308, D2S410 and a micro-satellite 5' to IL1R1 (D2S1473). D2S308 also produced a positive allele-wise ETDT result ($p = 0.0037$; $p_{\text{corr}} 0.02$), largely due to excess transmission of allele 4 (233 mu), which was transmitted 24 times and not transmitted eight times ($p = 0.0047$), as well as decreased transmission of allele 2 (229 mu) [transmitted 25 times and not transmitted 49 times ($p = 0.005$)]. Radiation hybrid mapping placed D2S308 and D2S363 at 651.5 cR and 654.7 cR, respectively from the telomere, i.e. within less than 1 cM of each other. Because these markers lie in close proximity, two-locus haplotypes of D2S363–D2S308 were also studied. Thirty different haplotypes were observed and these produced a significant result when tested with ETDT (empirical p value = 0.01). This was mainly accounted for by excess transmission of the following haplotypes; 363-3/308-2 (transmitted 16 times, not transmitted nine times), 363-3/308-3 (transmitted 13 times, not transmitted six times), 363-3/308-4 (transmitted ten times, not transmitted twice) and 363-4/308-4 (transmitted seven times, not transmitted once). However the large number of haplotypes makes it impossible to draw firm conclusions regarding which haplotypes specifically account for the overall positive ETDT results. None of the other markers used showed preferential allele transmission to diabetic offspring (Table 1).

The pairwise EH program was used to determine the linkage disequilibria between pairwise combination of markers. Strong disequilibrium was observed for two markers of the IL1R1 markers (D2S1473 and the *Pst*I RFLP) ($p < 0.00001$) and between the marker D2S160 and IL1A ($p = 0.007$); all other markers tested were found to be in complete linkage equilibrium with one another (Table 2).

Discussion

We have suggestive evidence that two closely-linked markers (D2S308 and D2S363) on chromosome 2q12–21 are associated with Type 1 diabetes susceptibility in the South Indian population; these markers have not been previously studied in other ethnic groups. A primary association with the IL-1 gene cluster or with the IL1R1 gene cannot explain the association in this ethnic group. Furthermore, various genome scans in white Caucasians have not implicated this region as a genetic susceptibility region for Type 1 diabetes, although the latter do not have the power to detect a locus with a low sibling risk (λ_s) which may only be found by association studies [2,24]. Replication studies in a larger cohort in the South Indian and in other ethnic groups are now indicated.

Many possibilities exist to explain the variable results for linkage and association studies between Type 1 diabetes and markers on 2q12–21. In particular, case control studies are prone to false positive or negative results due to hidden population stratification; in an effort to overcome these problems many researchers are now using family-based association studies [21,25]. Another factor affecting both linkage and association studies is that genetic and environmental determinants of Type 1 diabetes may differ between ethnic groups. In this regard, in the same families studied for markers on 2q12–21, we have shown a strong association of Type 1 diabetes and HLA-DQB1*0201 and -DQB1*0302 [26], although in a case control study in the same ethnic group we did not find an association with insulin gene (IDDM2) polymorphism [19].

The IL-1 gene cluster and IL1R1 have previously been studied, mainly using case-control studies, and gave inconsistent results [5,11–14,16–18]. The question therefore arises whether the association found between Type 1 diabetes and D2S308/D2S363 in the present study could be explained by linkage disequilibrium between these two markers and the IL-1 gene cluster and IL1R1, as previously examined. This possibility is unlikely for the following reasons. The report of the 4th International Workshop on Chromosome 2 [27] has suggested a marker order of: centromere-D2S160-IL1A-IL1B-IL1RN-D2S121-D2S308-telomere, with a distance of more than 3000 kb

between D2S160 and D2S308. The known distance between IL1RN and D2S308 accounts for more than half of this distance (nearly 1770 kb) [27], and our radiation hybrid mapping data places D2S363 a further 3.3 cR telomeric to D2S308 (up to 1000 kb) (data not shown). The IL1R1 gene is situated within the IL-1 receptor gene cluster, which has been mapped to the interval between D2S373 and D2S176, an area of about 2 cM on the genetic map [28]. This region is some 5 cM centromeric to the IL-1 cluster based on the same map data [28]. However, contig maps have put the marker D2S308 at a distance of 3000 kb to D2S160 [27]. Thus, based on both the physical distance and the projected map position of these markers, linkage disequilibrium is unlikely to exist between D2S308/363 and the IL1 gene cluster, and any disequilibrium certainly would not extend to the IL1R1 region. This is supported by the pairwise LD data in which linkage equilibrium was found between D2S308/363 and all other markers studied, including between D2S308 and D2S121, and in turn D2S121 with both IL1A and D2S160 (Table 2). The linkage disequilibrium observed in the South Indian subjects between D2S160 and IL1A encompasses more than 1000 kb of DNA. These results extend a previous study demonstrating linkage disequilibrium over 43 kb region of the IL1 gene cluster [11]. None of the markers studied, centromeric or telomeric to D2S308/D2S363, were associated with disease in this ethnic group, in agreement with other family studies which have failed to find overall linkage or association between Type 1 diabetes and the IL-1 gene cluster (sib-pair and TDT analysis) [2,5,11,24].

It is unclear why previous studies of European Caucasian patients [14,16,17] found an association with the IL1R1 locus whilst this and a previous study of South Indians did not find an association [17]. Case control studies of the Finnish DiMe samples have shown that non-HLA marker associations (IL1R1 and the insulin gene hypervariable region) are only detected after stratification by HLA-DRB genotype and are present only in non-HLA-DR3/non-DR4 individuals [17,29]. Furthermore, evidence of linkage between the IL1 cluster and rheumatoid arthritis has been found, but only after selecting families in which the affected siblings were discordant for both HLA DRB1 alleles [30]. In the South Indians a larger dataset would be required to investigate this hypothesis. Another possible explanation is that these previous case control studies represent false positives due to both hidden population stratification as well as the small numbers studied in the critical comparisons (i.e. the non-DR3/non-DR4 groups); indeed, a recent family association study in Denmark failed to confirm the case control studies in the same ethnic group [11].

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